HISTOLOGICAL ONTOGENY OF THE DIGESTIVE SYSTEM OF MARBLE GOBY (Oxyeleotris marmoratus) LARVAE

A. B. ABOL-MUNAFI, P. T. LIEM, M. V. VAN, M. A. AMBAK,
Institute of Tropical Aquaculture, Kolej Universiti Sains dan Teknologi Malaysia, 21030, Kuala Terengganu, Terengganu, Malaysia

A. W. M. EFFENDY
Center of Marine Biotechnology, Kolej Universiti Sains dan Teknologi Malaysia, Kuala Terengganu, Terengganu, Malaysia

M. AWANG SOH
Faculty of Agrotechnology and Food Science, Kolej Universiti Sains dan Teknologi Malaysia, 21030 Kuala Terengganu, Terengganu, Malaysia

Abstract: The development of the digestive system in the larvae of marble goby Oxyeleotris marmoratus was examined histologically from the 2nd to the 35th day after hatching. The larvae absorbed their yolk sacs 4-5 days after hatching, however they commenced exogenous feeding 2 days after hatching. After the onset of exogenous feeding 5 regions could be easily distinguished in the gut: the buccal cavity, the oesophagus, a future stomach, the intestine and the rectum. At this time, the intestinal tract was functional however the stomach was not developed completely. The first signs of intestinal absorption appeared in day 3 after hatching and could be identified as lipid vacuoles in the rectum. The larvae started to absorb protein in day 5. The stomach formed from day 10 to 15 but only circular muscle layer was identified in the muscularis externa. Liver and pancreas were formed and well-developed in the early larval period. After metamorphosis, the appearance of gastric glands in day 30 indicated that the development of digestive system was completed.

KEYWORDS: Oxyeleotris marmoratus, digestive system, exogenous feeding, functional stomach.

Introduction

Ingestion, digestion and assimilation of food are critical for the growth and survival of fish larvae. These processes are the main function of the digestive system which is one of the organ systems that has been studied histologically and physiologically in many fish species such as coregonid (Loewe and Eckmann, 1988), Dover sole (Boulhic and Gabaudan, 1992), African catfish (Verreth et al., 1992), gilthead seabream (Sarasquete et al., 1995), Siberian sturgeon (Gisbert et al., 1998), Atlantic halibut (Luizi et al., 1999), Nile tilapia (Tengjaroenkul et al., 2000, 2002), mud loach (Park and Kim, 2001), spotted sand bass (Pena et al., 2002). During larval period, the development of the digestive tract progress with larval growth, and efficiency of these processes also improve and hence result in improved larval survival and growth. Therefore, it is evident that a better knowledge of the digestive system and its functional capabilities is of great interest for progress in larval rearing techniques (Boulhic and Gabaudan, 1992; Bisbal and Bengtson, 1995; Sarasquete et al., 1995; Gisbert et al., 2004).

Correspondence: Ambok Bolong Abol-Munafi, Institute of Tropical Aquaculture, Kolej Universiti Sains dan Teknologi Malaysia, 21030 Kuala Terengganu, Terengganu, Malaysia

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Marble goby *Oxyeleotris marmoratus* is one of the most expensive table fish in Malaysia. It has been successfully spawned and reared under artificial conditions (Cheah et al., 1991; Senoo et al., 1994a; Abol-Munafi et al., 2002). However, high mortality during the larval stages is the major problem in mass production of this species. Starvation is the main reason causing high mortality of the larvae and it might be due to the limited mouth size (Liem et al., 1999) and incomplete digestive system. The preliminary results on morphological development of the mouth and sense organs of *O. marmoratus* larvae have been reported by Senoo et al. (1994b). However, information of alimentary system of the larvae was not obtained. The present study therefore describes the development of the major digestive organs of marble goby *O. marmoratus* larvae from hatching up to an age of 35 days.

**Materials and Methods**

Fish were obtained from natural spawning and reared in 4 m³ concrete tank at density of 5 fish/L. Larvae were fed with the mixture of live food organisms including rotifers, copepods and cladocerans. The green water from tilapia culture tanks containing mainly *Chlorella* and other phytoplankton species such as *Closterium, Scenedesmus, Coelastrum* were used to maintain the water quality. Temperature, DO and pH during the larval rearing ranged from 27.0 – 30.5°C, 3.8 – 7.2 mg/L and 7.2 – 7.8, respectively. Twenty fish were sampled immediately after hatching and on day 1, 2, 3, 5, 7, 10, 15, 20, 25, 30 and 35 after hatching following the changes in morphology and behaviour of marble goby *O. marmoratus* that has been reported by Senoo et al., (1994a; 1994b).

The histological methods employed followed Drury and Wallington (1967) and Kiernan (1990) methods. Fish were preserved in Bouin’s fixative solution for 6-12 hours or 10% neutral buffered formalin for at least 24 hours. Samples were transferred to 70% alcohol before tissue processing. After processing and embedding in paraffin wax, serial sections of 5 – 6 µm were made sagittally, stained and mounted. Staining was done by applying two methods: haematoxylin and eosin (HE), and periodic acid Schiff (PAS).

**Results**

**Digestive tract**

Just after hatching (AH), the digestive tract of *O. marmoratus* consisted of a straight undifferentiated tube, with a small lumen and closed oesophagus and anus. During the first 24 hours AH the gut differentiated into a buccal cavity, an oesophagus and an intestine. The anus remained closed. Larvae started exogenous feeding on day 2 AH, long before the yolk is used up (4-5 days AH). At this time, the digestive tract can be divided into 5 parts: buccopharynx, oesophagus, stomach, intestine and rectum (Fig. 1). Incipient liver and pancreas appeared as a group of round cells with spherical nucleus, prominent nucleolus and basophilic cytoplasm.

**Buccopharynx:** The buccopharynx was composed of a thin layer of stratified squamous epithelium which later consisted of mucous cells and taste buds on day 3 AH. A thin lamina propria underlies the oral mucosa. Striated muscles occurred in the tongue and in the pharynx. A pair of canine-like teeth appeared in the lower jaw on day 4 AH. On day 7 AH, two teeth in the upper jaw and 4 in lower jaw were observed. The teeth were arranged in a single row in the upper jaw on day 20 AH. Pharyngeal teeth grew on the dorsal surface of the epibranchial of the fourth gill arch and on the ventral surface of the ceratobranchial of the fifth gill arch.
Fig. 1: Longitudinal section of 2-day old larvae (HE, x10). Bc: buccal cavity; Y: yolk sac; L: liver; I: intestine; R: rectum; A: anus; Gb: gall bladder.

Fig. 2: Longitudinal section of the oesophagus of 30 days old larvae showing mucous cell Gc (PAS, x10)

Fig. 3: Longitudinal section of the “stomach” of 15-day old larvae showing the columnar epithelium cells Ce (HE, x40)

Fig. 4: Longitudinal section of the stomach of 30 days old larvae showing the gastric glands Gg (HE, x40)

Fig. 5: Longitudinal section of the intestine of 20 days old larvae showing the lipid vacuoles L (HE, x40)

Fig. 6: Longitudinal section of the rectum of 3-day old larvae showing fat accumulation (HE, x20). L: lipid vacuoles; R: rectum.

Oesophagus: The oesophagus differentiated on day 2 AH. It was layered with squamous epithelium cell in the early larval stage and started to fold on day 3 AH. The mucosa, which is longitudinally folded in the caudal region, was underlain by connective tissue. The changes during the larval development consisted of an increase in folding of the mucosa and in the number of mucous cells which were positive by PAS stained (Fig. 2). The transition between the oesophagus epithelium and the rest of digestive tract were well defined by the absence of mucous cells and the single layer of small prismatic epithelial cells of the digestive tract following the oesophagus. The strong muscular layer contained only striated muscle fibres and was divided into the inner longitudinal muscle (consisting of a ventral and dorsal bundle) and the outer circular muscle. The longitudinal muscle fibres appeared on day 35 AH.

Stomach: At the onset of exogenous feeding, the stomach was not present but the zone from which the stomach will be differentiated could be identified. It was orientated caudally in the prolongation of the oesophagus. The mucosa was observed to be composed only of a simple lining epithelium that was of the columnar type underlain by a thin and loose propria-submucosa (Fig. 3). The stomach was differentiated from intestine by a constriction of the digestive tract mucosa.

From day 10-15 AH, the stomach became more elongated and bent in its posterior region. Only circular muscle blocks were identified in the muscularis externa. The muscularis became two-layered as the longitudinal muscles appeared on day 30. At this time, the gastric glands were also identified (Fig. 4). The gastric gland that consisted of glandular cells first appeared in the lamina propria at the dorsal part of the cardiac portion. It then extended rapidly to ventral and posterior part, underlying the columnar cells except the pyloric portion.

Intestine: The intestine that was identified as a section between stomach and the ileorectal valve started to develop on day 1 AH. It consisted a single layer of columnar epithelial cells which resembled those of the undifferentiated stomach layered by thin serosa along its length. The epithelium started to fold on day 2 AH and the thickness of the epithelium cells increased with fish age. Lipid vacules were observed in the posterior intestine (Fig. 5) on day 7 AH. Only one layer of circular smooth muscle formed the muscularis externa. The intestinal mucosa showed very little variation during the course of metamorphosis.

Rectum: The rectum was separated from the anterior intestine by the ileorectal valve, which appeared in the larvae at 2 days AH. Organization of the rectal epithelium was similar to that of the intestine. Lipid vacules were observed in the rectum on day 3 AH, 24 hours after first feeding (Fig. 6). The rectal cells showed accumulation of protein from day 5 AH when small vacules appeared that were darkly-stained and positioned in the apical side of the enterocytes cytoplasm and were identified as proteinic vacules (Fig. 7).

Associated organs

Liver: The liver appeared as compact basophilic tissue and was situated between the yolk sac and the developing gut without any vacules. On day 3 AH, liver sinusoids became apparent. The hepatocytes morphology was more defined, with a big spherical and central nucleus, prominent nucleolus and a granular cytoplasm. Glycogen began to be stored in the liver as revealed by PAS reaction. Cell nucleus and cytoplasm were then pushed to the cell periphery by hepatocyte vacules.

Pancreas: The exocrine pancreas was arranged in acinar structure with well developed basophilic cells. From day 3 AH, the pancreatic duct appeared and the acidophilic zymogen granules could be seen within the pancreatic acini (Fig. 8). The pancreas started to enter the liver cells forming the hepatopancreas. Both liver and pancreas increased their size and complexity with larval development.

Discussion

During the larval stage, the development of the alimentary tract changed from a straight, undifferentiated gut to a complex and segmented digestive tract. At the first exogenous feeding, it was partially differentiated except for the stomach that was lacking. The liver and the pancreas differentiated and became functional. Structural alternations occurring during the larval stage were related to teeth, muscle layers and intestinal fold development. The results of present study were similar to those observed by Govoni et al., (1986), Bisbal and Bengtson (1995), and Luizi et al., (1999), Park and Kim (2001) and Pena et al., (2002) in other fish species.

The early development of oesophagus in *O. marmoratus* larvae might be important at the onset of first feeding. The increase of mucous cells and then the mucus might facilitate the movement of food, and the mucosal folds would allow for distension during food consumption. This agreed with the finding in Dover sole *Solea solea* (Bouhlic and Gabaudan, 1992) and sea bass *Sparus aurata* (Sarasquete et al., 1995). In *O. marmoratus* the PAS positive reaction of the mucous cells indicated they contained at least neutral mucus substances.

At the first feeding, the larvae seemed to compensate for the lack of gastric pre-digestion by active intracellular digestion in the rectal epithelial cells following the pinocytosis of macromolecules from the lumen. This mechanism of digestion is well known to occur in many other larval teleosts (Govoni et al., 1986; Loewe and Eckmann, 1988; Sarasquete et al., 1995; Luizi et al., 1999). The histological evidence for this process was the presence of supranuclear inclusions containing acidophilic granules in the hindgut epithelial cells. Indeed, such inclusions were observed in the rectum of *O. marmoratus* on day 5 AH. These inclusions disappeared when the stomach became functional and led to extracellular digestion.

In the present investigation, active lipid digestion was confirmed by the appearance of large lipid vacuoles of the mucosal epithelial cells in both the intestine and rectum. Large lipid droplets were thought to be temporary storage (Govoni, 1986). Loewe and Eckmann (1988) supposed the increase of number of lipid vacuoles might be due to the fact the larvae were easily to build up the storage form (the supranuclear) but difficulty in building up the transport form (the chylomicrons) during a certain period of the digestive development. Generally, the large supranuclear vacuoles of the midgut corresponded to absorption of lipid while the acidophilic granular inclusions of the hindgut represented pinocytotic protein absorption (Govoni et al., 1986). In case of *O. marmoratus*, lipid vacuoles and proteinic inclusions were observed in the rectum 1 day and 3 days after first feeding, respectively. However, from start of feeding to day 7 AH most lipid drops appeared in the rectum and the lipid vacuoles were only observed in the posterior intestine after day 7. This suggests that the larvae have poor intestinal lipid digestion in the first few days of exogenous feeding. Furthermore, in the short larval gut the retention time remained too short to involve the intestinal enterocytes.

In marble goby *O. marmoratus*, the gastric glands started to function and secreted gastric enzymes just after it was formed (day 30 AH). It can be observed by the appearance of the PAS positive mucous layer. Probably, it protects the stomach wall from auto-digestion by the HCl and enzymes produced by the gastric glands. This finding was in agreement with the results in African catfish *Clarias gariepinus* (Verreth et al., 1992) and in rainbow trout *Oncorhynchus mykiss* (Ostos Garrido et al., 1993).

Due to the lack of stomach, liver and pancreas were believed to be the sources of digestive enzymes that contributed to the digestive and absorptive process in the intestine of the.
laurae during the course of metamorphosis. Liver was likely the source of esterase (Hirji and Courney, 1983) and lipase secretion (Govoni et al., 1986). The acinar cells of the pancreas consisted of acidophilic zymogen granules, presumably the precursors of trypsin and chymotrypsin (Govoni et al., 1986). In O. marmoratus, the appearance of numerouszymogens in the pancreatic acinar cells and the hepatocyte vacuoles in hepatocytes confirmed the function of these organs.

With an incomplete digestive system, the marble goby larvae would have to rely on a food source that: (i) was at least partially and easily digestible, (ii) contained enzymes systems which allow autolysis, and (iii) supplied in abundance all the essential nutrients required by the larvae (Lawens and Sorgeles, 1996). Thus, live food organisms seemed to meet all the necessary criteria for the larvae during larval stage.

Conclusion

During the metamorphosis stage, marble goby larvae possessed a short digestive tract without a functional stomach, and a few functional enzyme systems as liver and pancreas. Larval stage of this species ended at day 30 AH that confirmed by the appearance of gastric glands in the stomach. Beside of lacking stomach, the larvae have problem with digestion and absorption of food in the first few days of exogenous feeding.

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